Structure, Vol. 12, January, 2004, ©2004 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.str.2003.12.009

A New Catalytic Dyad Regulates Anchoring of Molecules to the Gram-Positive Cell Wall by Sortases

In this issue of *Structure*, three crystal structures of staphylococcal sortase B reveal a new active site for cysteine proteases, which requires a Cys-Arg catalytic dyad for substrate turnover.

The cell wall of Gram-positive pathogens provides an optimal scaffold for the attachment of a variety of surface-exposed proteins, a number of which play key roles in virulence and are essential for a successful infection process. Roles played by such molecules differ widely and range from adhesion to the host cytoskeletal framework (such as fibronectin binding proteins; FnbA/FnbB in *Staphylococcus aureus*, PavA in *Streptococcus pneumoniae*) to recognition of molecules involved in the immune response and proteolysis of host macromolecules (such as CbpA and CbpG of *S. pneumoniae*). Their stable anchoring to the exposed surface of the bacterial cell wall, then, determines the bacterium's ability to invade deep tissues and evade host defense, and may be performed by any of five distinct mechanisms: association through a membrane anchor, recognition of choline groups on (lipo) teichoic acids through choline binding domains, direct association to lipoteichoic acids, cysteinyl lipoylation, or covalent achoring of an LPXT motif to the peptidoglycan through the action of sortases. In recent years, Schneewind and colleagues have provided details of this latter mechanism, as well as evidence that sortase anchoring is crucial for virulence in *S. aureus* (Mazmanian et al., 1999, 2000). In this issue of *Structure*, the crystal structures of the newly identified sortase B in complex with two active site inhibitors and a cell wall substrate analog have identified the unique catalytic machinery employed by sortases, thus shedding light on possibilities for novel anti-infective development through the targeting of this uncanny active site.

Sortase A acts both as a cysteine protease and a transpeptidase, cleaving protein precursor molecules at the LPXTG motif and crosslinking the free C-terminal to the amino group of pentaglycine cross-bridges on the bacterial peptidoglycan (Ton-That et al., 2000). This reaction is reminiscent of the one catalyzed by penicillin binding proteins (PBPs), which participate in peptidoglycan crosslinking (and thus structuring) by cleavage of a D-Ala-D-Ala moiety of a peptide chain, formation of an acyl-enzyme complex, and reaction of the intermediate with the amino group from a neighboring chain (Di Guilmi et al., 2003). The NMR structure of sortase A (Ilangovan et al., 2001) revealed an active site with some similarity to that of proteins from the papain/cathepsin families, in which the nucleophilic Cys is held in an active configuration through a thiolate-imidazolium ion interaction with a nearby His residue. In the case of sortase A, Cys184 was known to be the active site nucleophile through mutagenesis and modification with thiol-targeting compounds (Ton-That and Schneewind, 1999), but His 120 was suggested as being the residue responsible for the facilitation of thiolate formation mostly due to its conservation in a variety of compared sortase sequences. The structure showed, however, that the two side chains not only did not interact, but pointed away from each other (Ilangovan et al., 2001). Interestingly, studies of pH dependence of sortase A by an irreversible inhibitor, as well as NMR analyses, prompted Clubb and coworkers to suggest that the active site of sortase A did not have a thiolate-imidazolium ion pair (Connolly et al., 2003).

The three crystal structures of sortase B in this issue of *Structure* (Zong et al.) not only clarify the discrepancy but also reveal a novel mechanism for cysteine protease catalysis. Sortase B cleaves the IsdC heme-iron transport protein at an NPQTN motif (and is thus much more specific than sortase A, which targets a plethora of adhesion and virulence-related molecules). The structures of the active site of sortase B complexed with sulfhydryl-targeting inhibitors clearly show that the molecules bind to the active site cysteine (Cys 233), but are both too far from His 130 to involve the latter residue in participation in the reaction. A much more likely ionizable candidate is Arg 233, which is conserved in all sortases in Gram-positive microorganisms. Notably, in the crystal structure of sortase B complexed to triple-glycine, which emulates the peptidoglycan cross-bridge moiety, the guanidinium group of Arg 233 is optimally positioned to fulfill the role of deprotonation of the substrate N-terminal group.

Well-known cysteine proteases, such as papain and cathepsins, contain a Cys-His-Asn catalytic triad where Cys/His exist, in the "resting state" of the enzyme, as a thiolate/imidazolium pair. Since thiolate is an extremely powerful nucleophile, the first step in catalysis does not require much energy. The conservation of the Cys-Arg motif throughout sortase sequences from a variety of different Gram-positive pathogens strongly points to the universal employment of this novel residue pair in sortase catalysis. The exact catalytic mechanism, however, remains to be determined and is an interesting enzymological question.

The structural and biochemical characterization of both staphylococcal sortases now reveals that distinct sortases, expressed in the same microorganism, are probably necessary in order to process different substrates; the conservation of active site residues, however, suggests that they may all proceed via the same catalytic mechanism. This is a crucial finding, given the essential role that such proteins play in virulence factor targeting to the bacterial cell wall. Consequently, exploitation of this uncommon active site for potential drug development may be one of the most promising efforts in the search for new ways to control microbial pathogenesis and infection.

Andréa Dessen
Institut de Biologie Structurale (CNRS/CEA/UJF)
41 rue Jules Horowitz
38027 Grenoble
France

Selected Reading

Connolly, K.M., Smith, B.T., Pilpa, R., Ilangovan, U., Jung, M.E., and Clubb, R.T. (2003). J. Biol. Chem., in press.

Di Guilmi, A.M., Dessen, A., Dideberg, O., and Vernet, T. (2003). J. Bacteriol. *185*, 1650–1658.

Ilangovan, U., Ton-That, H., Iwahara, J., Schneewind, O., and Clubb, R. (2001). Proc. Natl. Acad. Sci. USA *98*, 6056–6061.

Mazmanian, S.K., Liu, G., Ton-That, H., and Schneewind, O. (1999). Science *285*, 760–763.

Mazmanian, S.K., Liu, G., Jensen, E.R., Lenoy, E., and Schneewind, O. (2000). Proc. Natl. Acad. Sci. USA *97*, 5510–5515.

Ton-That, H., and Schneewind, O. (1999). J. Biol. Chem. *274*, 24316–24320.

Ton-That, H., Mazmanian, H., Faull, K.F., and Schneewind, O. (2000). J. Biol. Chem. *275*, 9876–9881.

Zong, Y., Mazmanian, S.K., Schneewind, O., and Narayana, S.V.L. (2004). Structure *12*, this issue, 105–112.

Structure, Vol. 12, January, 2004, DOI 10.1016/j.str.2003.12.007

Studying Topoisomerases in the Fourth Dimension

Two recent studies on DNA topoisomerases combine the power of biochemistry and structural biology to gain new insight at the atomic level into how these enzymes interact with DNA (Perry and Mondragón, 2003 [Structure, Nov. 2003]; Tian et al., 2004 [this issue]).

It is an important fact of life that DNA is flexible. Thus it can withstand the extreme compaction necessary to pack inside living cells, as well as the torsional strain that arises during processes like transcription and replication. In response to these stresses, the double-helical axis of DNA coils in space, a process termed supercoiling. Cells rely on enzymes called topoisomerases to remove excessive supercoiling while maintaining sufficient supercoiling to ensure a stably compact genome.

Topoisomerases participate in nearly every cellular process that involves DNA, including transcription, replication, recombination, and chromosome segregation (reviewed in Champoux, 2001). All topoisomerases use a three-step mechanism of cleavage, strand passage, and religation. In the first step, a nucleophilic tyrosine attacks the phosphodiester backbone, cleaving the DNA and creating a covalent intermediate of protein-DNA termed the "cleavage complex." Type I topoisomerases cleave one strand of the duplex, whereas type II enzymes cleave both strands. Type I enzymes are further divided into type IA and IB, depending on whether they attach to the 5′ or 3′ phosphate of the DNA, respectively. In the cleavage complex, the free ends of the cleaved strand(s) are separated and the other strand of the duplex (in the type I case) or another region of duplex (in the type II case) passes through this gap. Finally the backbone of the cleaved DNA is religated and the duplex is released.

Biochemical studies proved powerful enough to provide us with the general mechanism of topoisomerases, but only by combining biochemistry with X-ray crystallography have we come to realize that in carrying out their three-step mechanism, topoisomerases undergo various conformational changes as they pass through a series of transient intermediate states. Two recent reports reveal new details about this pathway for type IA and type IB enzymes. Perry and Mondragón (2003) determined the structure of a type IA enzyme complexed to single-stranded DNA and provide structural evidence for a new conformational intermediate. Tian and coworkers examine the interactions of a type IB enzyme with modified DNA substrates to provide evidence that protein contacts with the phosphate backbone drive conformational changes in the enzyme. Both papers address how enzyme and DNA contact each other, and how these contacts change over the course of the reaction.

Topoisomerases I and III from *E. coli* show a similar padlock-shaped structure bearing a central hole, which is thought to take up and hold the passed DNA strand until after religation (Lima et al., 1994). The structure of topoisomerase III complexed with a single-stranded octanucleotide (Changela et al., 2001) provided the first glimpse of a type IA enzyme bound to DNA (Figure 1A). The structure revealed considerable conformational changes in the protein relative to the apoenzyme (Figure 1B), including a reorientation of the active site pocket to bring the catalytic tyrosine into position to attack the scissile bond. This conformation is thought to correspond to a point in the reaction pathway just before cleavage or just after religation. The more recent structure of topoisomerase I complexed with a single-stranded oligonucleotide (Perry and Mondragón, 2003) provides direct evidence for another transient intermediate. In contrast to the topoisomerase III complex, this structure is almost unchanged from that of the apoenzyme. The active site pocket is not positioned for catalysis, suggesting that this conformation represents an intermediate just after the initial DNA binding or just prior to its release.

These crystal structures, together with elegant biochemical data (Li et al., 2001), provide strong evidence that type IA topoisomerases use a multistep pathway to cleave one DNA strand and pass the other into the central hole of the enzyme. An important goal now is to